CLAIMS

What is claimed is:

1. A method of enriching an expression library comprising DNA encoding a protein involved in insulin stimulated GLUT4 trafficking at the plasma membrane comprising:
   a) sorting cells containing an expression library comprising DNA encoding a protein involved in insulin stimulated GLUT4 trafficking for cells with an altered proportion of GLUT4 at the cell surface;
   b) expanding the sorted cells from step a) in culture;
   c) sorting the cells expanded in step b); and
   d) expanding the cells sorted in step c);

   thereby forming an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane.

2. The method of Claim 1, wherein the cells containing the expression library respond to insulin stimulation by externalizing GLUT4 in a biphasic pattern.

3. The method of Claim 1, wherein the cells with an altered proportion of GLUT4 at the cell surface are identified about five minutes after insulin stimulation.

4. The method of Claim 1, wherein the proportion of GLUT4 at the cell surface is increased.

5. The method of Claim 1, wherein the proportion of GLUT4 at the cell surface is decreased.

6. The method of Claim 1, wherein the method comprises additional steps of sorting the cells and expanding the sorted cells in culture.

7. The method of Claim 6, wherein in one sorting step individual cells are sorted for expansion.

8. The method of Claim 1, wherein the cells were cultured in a media with high amino acid content.

9. The method of Claim 8, wherein the media is Dulbecco's modified Eagle's medium (DMEM).

10. The method of Claim 1, wherein the cells are selected from the group of adipocyte, fibroblast and muscle cells.

11. The method of Claim 1, wherein the cells are 3T3-L1 or Chinese Hamster Ovary (CHO) cells.

12. The expression library prepared in the method of Claim 1.

13. The expression library prepared in the method of Claim 5.

14. The expression library prepared in the method of Claim 6.

15. A method for identifying a protein involved in insulin stimulated GLUT4 trafficking at the plasma membrane comprising:

a) introducing an expression library comprising DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane into a population of cells;

b) maintaining the population of cells under conditions suitable for replication of the DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane and for isolating individual clones;

c) subdividing the population of cells into pools of cells such that each pool is a subset of the population of cells;

d) isolating the replicated DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane from the pools of cells;

e) introducing the replicated DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane into cells containing a GLUT4 reporter protein; and

(f) assessing trafficking of the reporter protein,

wherein altered trafficking of the reporter protein upon insulin stimulation is indicative of the presence of a protein involved in GLUT4 trafficking at the plasma membrane.

16. The method of Claim 15, further comprising repeating steps c) - f) for the pools of cells containing a protein involved in GLUT4 trafficking at the plasma membrane.

17. The method of Claim 15, wherein the cells containing the expression library respond to insulin stimulation by externalizing GLUT4 in a biphasic pattern.

18. The method of Claim 15, wherein the protein is associated with an increased proportion of GLUT4 at the plasma membrane.

19. The method of Claim 15, wherein the protein is associated with a decreased proportion of GLUT4 at the plasma membrane.

20. The method of Claim 15, wherein the cells of the expression library are selected from the group of adipocyte, fibroblast and muscle cells.

21. The method of Claim 15, wherein the cells of the expression library are 3T3-L1 or CHO cells.

22. A protein identified in the method of Claim 15.

23. The protein of Claim 22 comprising a UBX domain.

24. The protein of Claim 22 comprising a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

25. A method for identifying a protein involved in GLUT4 trafficking at the plasma membrane comprising the following steps:

   a) preparing an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane in cells;
   b) stimulating the cells with insulin; and
   c) screening the cells for altered GLUT4 trafficking at the plasma membrane;

wherein altered GLUT4 trafficking upon insulin stimulation is indicative of the presence of a protein involved in GLUT4 trafficking at the plasma membrane.

26. The method of Claim 25, wherein the cells respond to insulin stimulation by externalizing GLUT4 in a biphasic pattern.

27. The method of Claim 25, wherein the cells are screened by identifying those cells with altered proportions of GLUT4 at the cell surface about five minutes after insulin stimulation.

28. The method of Claim 25, wherein the expression library is enriched for DNA associated with an increased proportion of GLUT4 at the plasma membrane.

29. The method of Claim 25, wherein the expression library is enriched for DNA associated with a decreased proportion of GLUT4 at the plasma membrane.

30. The method of Claim 25, wherein the cells are selected from the group of adipocyte, fibroblast and muscle cells.

31. The method of Claim 25, wherein the cells are 3T3-L1 or CHO cells.

32. A protein identified in the method of Claim 25.

33. The protein of Claim 32 comprising a UBX domain.

34. The protein of Claim 32 comprising a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

35. An isolated antibody or antigen binding fragment thereof which specifically binds to a protein selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

36. The antibody of Claim 35, wherein the antibody is a monoclonal antibody.

37. The antibody of Claim 35, wherein the antibody is a polyclonal antibody.

38. A method of identifying an agent which alters insulin stimulated GLUT4 trafficking at the plasma membrane comprising the steps of:

    a) maintaining test cells in which GLUT4 is transferred to the cell surface upon stimulation with insulin in the presence of an agent which binds to a UBX domain;

    b) measuring the proportion of GLUT4 at the cell surface of the test cells after insulin stimulation; and

    c) comparing the proportion of GLUT4 at the cell surface with the proportion of GLUT4 at the cell surface in suitable control cells after insulin stimulation,

    wherein an altered proportion of GLUT4 at the cell surface in the test cells compared to the proportion of GLUT4 in the control cells is indicative of altered insulin stimulated GLUT4 trafficking at the plasma membrane.

39. The method of Claim 38, wherein GLUT4 trafficking at the plasma membrane is increased in the presence of the agent.

40. The method of Claim 38, wherein GLUT4 trafficking at the plasma membrane is decreased in the presence of the agent.

41. The method of Claim 38, wherein the agent binds to a protein comprising a sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

42. The method of Claim 38, wherein the agent binds to L1.

43. The method of Claim 38, wherein the cells are selected from the group of adipocyte, fibroblast and muscle cells.

44. The method of Claim 38, wherein the cells are 3T3-L1 or CHO cells.

45. A method for identifying an agent which binds to a protein comprising a UBX domain comprising the steps of:

a) isolating a protein comprising a UBX domain;

b) contacting an agent with the isolated protein under conditions suitable for binding of the agent to the isolated protein; and

c) detecting a resulting agent-protein complex,

wherein detection of an agent-protein complex is indicative of an agent which binds to the protein.

46. The method of Claim 45, wherein the agent binds to a protein comprising a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

47. A method of identifying an agent which alters the interaction between GLUT4 and L1 comprising:

a) comprising combining GLUT4, L1 and an agent under conditions appropriate for interaction between GLUT4 and L1

b) determining the extent to which GLUT4 and L1 interact; and

c) comparing the extent of the GLUT4-L1 interaction in the presence of the agent with the extent of GLUT4-L1 interaction in the absence of the agent,

whereby if the extent of the GLUT4-L1 interaction differs significantly in the presence of the agent when compared to the interaction in the absence of the agent, then the candidate agent is one which alters interaction between GLUT4 and L1.

48. The method of Claim 47, wherein the agent enhances the interaction.

49. The method of Claim 47, wherein the agent inhibits the interaction.

50. A method of altering insulin stimulated GLUT4 trafficking comprising contacting an insulin responsive cell with rapamycin.

51. A method of altering insulin stimulated GLUT4 trafficking comprising contacting a cell with an agent which binds to the UBX domain of a protein selected from the selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

52. A method of inhibiting GLUT4 externalization comprising contacting a GLUT4/L1 complex with an agent that inhibits disassociation of the complex.

53. A method of enhancing GLUT4 externalization comprising contacting a GLUT4/L1 complex with an agent that enhances disassociation of the complex.

54. A method of enriching an expression library comprising DNA encoding a protein involved in insulin stimulated GLUT4 trafficking at the plasma membrane comprising sorting cells containing an expression library comprising DNA encoding a protein involved in insulin stimulated GLUT4 trafficking for cells with an altered proportion of GLUT4 at the cell surface, thereby forming an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking at the plasma membrane.

55. The method of Claim 54, wherein the sorted cells are expanded in culture.

56. The method of Claim 55 further comprising additional steps of sorting expanded cells and expanding the sorted expanded cells in culture until a desired level of enrichment is achieved.

57. A method of enriching an expression library comprising DNA encoding a protein involved GLUT4 trafficking comprising:

a) sorting cells containing an expression library comprising DNA encoding a protein involved in insulin stimulated GLUT4 trafficking for cells with an altered proportion of GLUT4 at the cell surface;

b) expanding the sorted cells from step a) in culture;

c) sorting the cells expanded in step b); and

d) expanding the cells sorted in step c);

thereby forming an expression library enriched for DNA encoding a protein involved in GLUT4 trafficking.